

CLAIMS

We claim:

1. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising:

5 a) providing a first probe comprising:

- i) an upstream universal priming site (UUP);
- ii) an adapter sequence;
- iii) a first target-specific sequence comprising a first base at a readout position; and
- iv) a downstream universal priming site (DUP);

10 b) contacting said first probe with said target sequence under conditions whereby only if said first base is perfectly complementary to a nucleotide at said detection position is a first hybridization complex formed;

15 c) removing non-hybridized first probes;

20 d) denaturing said hybridization complex;

25 e) amplifying said first probe to generate a plurality of amplicons;

30 f) contacting said amplicons with an array of capture probes; and

35 g) determining the nucleotide at said detection position.

2. A method according to claim 1 wherein said amplicons comprise a label.

3. A method according to claim 1 further comprising:

40 a) providing a second probe comprising:

- i) an upstream universal priming site (UUP);
- ii) an adapter sequence;
- iii) a second target-specific sequence comprising a second base at said readout position; and
- iv) a downstream universal priming site (DUP);

45 b) contacting said second probe with said target sequence under conditions whereby only if said second base is perfectly complementary to a nucleotide at said detection position is a second hybridization complex formed;

50 c) removing non-hybridized second probes;

55 d) denaturing said second hybridization complex;

- e) amplifying said second probe to generate a plurality of amplicons;
- f) contacting said amplicons with an array of capture probes; and
- g) determining the nucleotide at said detection position.

4. A method of determining the identification of a nucleotide at a detection position in a target
5 sequence comprising:

- a) providing a plurality of readout probes each comprising:
 - i) an upstream universal priming site (UUP);
 - ii) an adapter sequence;
 - iii) a target-specific sequence comprising a unique base at a readout
10 position; and
 - iv) a downstream universal priming site (DUP);
- b) contacting said detection probes with said target sequence under conditions
15 whereby only if said base at said readout position is perfectly complementary to a
nucleotide at said detection position is a first hybridization complex formed;
- c) removing non-hybridized first probes;
- d) denaturing said first hybridization complex;
- e) amplifying said detection probes to generate a plurality of amplicons;
- f) contacting said amplicons with an array of capture probes; and
- g) determining the nucleotide at said detection position.

20 5. A method of determining the identification of a nucleotide at a detection position in a target
sequence comprising a first target domain comprising said detection position and a second target
domain adjacent to said detection position, wherein said method comprises:

- a) hybridizing a first ligation probe to said first target domain, said first ligation probe
comprising:
 - i) an upstream universal priming site (UUP); and
 - ii) a first target-specific sequence; and
- b) hybridizing a second ligation probe to said second target domain, said second
ligation probe comprising:
 - i) a downstream universal priming site (DUP); and
 - ii) a second target-specific sequence comprising a first base at an
interrogation position;

30 wherein if said first base is perfectly complementary to said nucleotide at said

detection position a ligation complex is formed and wherein at least one of said first and second ligation probes comprises an adapter sequence;

5 c) removing non-hybridized first probes;

 d) providing a ligase that ligates said first and second ligation probes to form a ligated probe;

 e) amplifying said ligated probe to generate a plurality of amplicons;

 f) contacting said amplicons with an array of capture probes; and

 g) determining the nucleotide at said detection position.

6. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, wherein said method comprises:

10 a) hybridizing a first ligation probe to said first target domain, said first ligation probe comprising:

 i) an upstream universal priming site (UUP); and

 ii) a first target-specific sequence; and

 b) hybridizing a second ligation probe to said second target domain, said second ligation probe comprising:

 i) a downstream universal priming site (DUP); and

 ii) a second target-specific sequence comprising a first base at an interrogation position;

 wherein if said first base is perfectly complementary to said nucleotide at said detection position a ligation complex is formed and wherein at least one of said first and second ligation probes comprises an adapter sequence;

 c) removing non-hybridized first probes;

 d) providing a ligase that ligates said first and second ligation probes to form a ligated probe;

25 e) hybridizing said ligated probe to a rolling circle (RC) sequence comprising:

 i) an upstream priming sequence; and

 ii) a downstream priming sequence;

 f) providing a ligase that ligates said upstream and downstream priming sites to form a circular ligated probe;

 g) amplifying said circular ligated probe to generate a plurality of amplicons;

 f) contacting said amplicons with an array of capture probes; and

 g) determining the nucleotide at said detection position.

7. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, wherein said method comprises:

5 a) hybridizing a rolling circle (RC) probe to said target sequence, said RC probe

comprising:

- i) an upstream universal priming site (UUP); and
- ii) a first target-specific sequence;
- iii) a second target-specific sequence comprising a first base at an interrogation position; and
- iv) an adapter sequence;

10 wherein if said first base is perfectly complementary to said nucleotide at said detection position a ligation complex is formed;

15 c) providing a ligase that ligates said first and second ligation probes to form a ligated probe;

d) amplifying said ligated probe to generate a plurality of amplicons;

e) contacting said amplicons with an array of capture probes; and

f) determining the nucleotide at said detection position.

8. A method according to claim 7, further comprising removing non-hybridized RC probe.

9. A method according to claim 1, 4, 5, 6 or 8 wherein said removing comprises:

20 a) enzymatically adding a binding ligand to said target sequence;

b) binding a hybridization complex comprising said target sequence comprising said binding ligand to a binding partner immobilized on a solid support;

c) washing away unhybridized probes; and

d) eluting said probe off said solid support.

25 10. A method according to claim 1, 4, 5, 6 or 8 wherein said removing is done using a double-stranded specific moiety.

11. A method according to claim 10 wherein said double-stranded specific moiety is an intercalator attached to a support.

12. A method according to claim 9 wherein said support is a bead.

13. A method according to claim 1, 4, 5, 6 or 7 wherein said amplifying is done by:

- hybridizing a first universal primer to said UUP;
- providing a polymerase and dNTPs such that said first universal primer is extended;
- hybridizing a second universal primer to said DUP;
- providing a polymerase and dNTPs such that said second universal primer is extended; and
- repeating steps a) through d).

14. A method according to claim 1, 4, 5, 6 or 7 wherein said array comprises:

- a substrate with a patterned surface comprising discrete sites; and
- a population of microspheres comprising at least a first subpopulation comprising a first capture probe and a second subpopulation comprising a second capture probe.

15. A method according to claim 14 wherein said discrete sites comprise wells.

16. A method according to claim 14 or 15 wherein said substrate comprises a fiber optic bundle.

17. A method of determining the identification of a nucleotide at a detection position in a genomic target sequence comprising:

- attaching a library of genomic target sequences to a solid support;
- adding at least one probe and an enzyme to form an extended primer;
- denaturing said extended primer from said target sequence;
- hybridizing said extended primer to an array comprising capture probes; and
- determining said nucleotide at said detection position.

18. A method according to claim 17, further comprising removing unhybridized probes.

19. A method according to claim 1, 4, 5, 6 or 7, further comprising providing a support on which the target sequence is immobilized.

20. A method according to claim 19, wherein said non-hybridized first probes are removed without

removing said target sequence from said support.

21. A method according to claim 1, 4, 5, 6 or 7, further comprising attaching said target sequence to a support.
22. A method according to claim 21, wherein said target sequence is attached to said support by a method selected from the group consisting of labeling said target sequence with a functional attachment moiety, absorption of said target sequence on a charged support, direct chemical attachment of said target sequence to said support and photocrosslinking said target sequence to said support.
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23. A method according to claim 1, 4, 5, 6 or 7, wherein said support is selected from the group consisting of paper, plastic and tubes.
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24. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising:
 - a) providing a support on which the target sequence is immobilized;
 - b) providing a first probe comprising:
 - i) an upstream universal priming site (UUP);
 - ii) an adapter sequence;
 - iii) a first target-specific sequence comprising a first base at a readout position; and
 - iv) a downstream universal priming site (DUP);
 - c) contacting said first probe with said target sequence under conditions whereby only if said first base is perfectly complementary to a nucleotide at said detection position is a first hybridization complex formed;
 - d) removing non-hybridized first probes;
 - e) denaturing said hybridization complex;
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 - f) amplifying said first probe to generate a plurality of amplicons;
 - g) contacting said amplicons with an array of capture probes; and
 - h) determining the nucleotide at said detection position
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25. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising:
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a) providing a support on which the target sequence is immobilized;

b) providing a plurality of readout probes each comprising:

- i) an upstream universal priming site (UUP);
- ii) an adapter sequence;
- iii) a target-specific sequence comprising a unique base at a readout position; and
- iv) a downstream universal priming site (DUP);

c) contacting said detection probes with said target sequence under conditions whereby only if said base at said readout position is perfectly complementary to a nucleotide at said detection position is a first hybridization complex formed;

d) removing non-hybridized first probes;

e) denaturing said first hybridization complex;

f) amplifying said detection probes to generate a plurality of amplicons;

g) contacting said amplicons with an array of capture probes; and

h) determining the nucleotide at said detection position.

26. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, wherein said method comprises:

- a) providing a support on which the target sequence is immobilized;
- b) hybridizing a first ligation probe to said first target domain, said first ligation probe comprising:
 - i) an upstream universal priming site (UUP); and
 - ii) a first target-specific sequence; and
- c) hybridizing a second ligation probe to said second target domain, said second ligation probe comprising:
 - i) a downstream universal priming site (DUP); and
 - ii) a second target-specific sequence comprising a first base at an interrogation position;

wherein if said first base is perfectly complementary to said nucleotide at said detection position a ligation complex is formed and wherein at least one of said first and second ligation probes comprises an adapter sequence;

d) removing non-hybridized first probes;

e) providing a ligase that ligates said first and second ligation probes to form a ligated probe;

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- f) amplifying said ligated probe to generate a plurality of amplicons;
- g) contacting said amplicons with an array of capture probes; and
- h) determining the nucleotide at said detection position.

27. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, wherein said method comprises:

- a) providing a support on which the target sequence is immobilized;
- b) hybridizing a first ligation probe to said first target domain, said first ligation probe comprising:
 - i) an upstream universal priming site (UUP); and
 - ii) a first target-specific sequence; and
- c) hybridizing a second ligation probe to said second target domain, said second ligation probe comprising:
 - i) a downstream universal priming site (DUP); and
 - ii) a second target-specific sequence comprising a first base at an interrogation position;

wherein if said first base is perfectly complementary to said nucleotide at said detection position a ligation complex is formed and wherein at least one of said first and second ligation probes comprises an adapter sequence;

- d) removing non-hybridized first probes;
- e) providing a ligase that ligates said first and second ligation probes to form a ligated probe;
- f) hybridizing said ligated probe to a rolling circle (RC) sequence comprising:
 - i) an upstream priming sequence; and
 - ii) a downstream priming sequence;
- g) providing a ligase that ligates said upstream and downstream priming sites to form a circular ligated probe;
- h) amplifying said circular ligated probe to generate a plurality of amplicons;
- i) contacting said amplicons with an array of capture probes; and
- j) determining the nucleotide at said detection position.

28. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, wherein said method comprises:

a) providing a support on which the target sequence is immobilized;

b) hybridizing a rolling circle (RC) probe to said target sequence, said RC probe comprising:

5 i) an upstream universal priming site (UUP); and

ii) a first target-specific sequence;

iii) a second target-specific sequence comprising a first base at an interrogation position; and

iv) an adapter sequence;

10 wherein if said first base is perfectly complementary to said nucleotide at said detection position a ligation complex is formed;

c) providing a ligase that ligates said first and second ligation probes to form a ligated probe;

d) amplifying said ligated probe to generate a plurality of amplicons;

e) contacting said amplicons with an array of capture probes; and

15 f) determining the nucleotide at said detection position.

29. A method according to claim 28, further comprising removing unhybridized RC probe.